Canad. M. A. J.
Dec. 1951, vol. 65

FOLIC ACID ANTAGONISTS IN THE TREATMENT OF LEUKÆMIA IN CHILDREN

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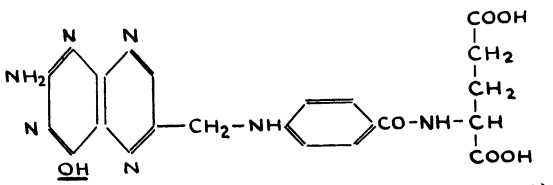
FOLIC ACID (pteroyl-glutamic acid) is a vitamin factor essential to practically all forms of life. This substance has been isolated in a chemically pure form and synthesized. A number of conjugates or analogues with chemical structures similar to folic acid have been synthesized. Some of these have the same properties as folic acid in regard to promoting the growth of certain test bacteria (*Lactobacillus casei* and *Streptococcus fecalis* R.). Others are biologically antagonistic to folic acid and inhibit its growth-promoting qualities. The most powerful of these folic acid antagonists is 4-amino-pteroyl-glutamic acid (aminopterin). Other conjugates of folic acid which are less antagonistic, are a-methopterin, amino-an-fol, an-fol-A, and met-fol-B. The formulæ and chemical structure of the various folic acid analogues are given by Farber.[1]

In Fig. 1, the structural formulæ of folic acid and aminopterin are shown. It may be seen at a glance how similar are the chemical configurations of these two compounds and yet biologically they are so diametrically opposite in their effect on living cells. Although the action of these antagonists is not completely understood it is assumed, because of their chemical similarity to folic acid that they are able to block an essential metabolic process in which folic acid is a necessary component.



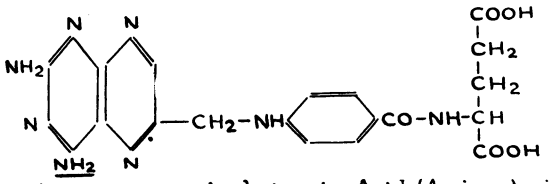


Fig. 1

Farber,[2] working with folic acid conjugates, biologically similar in their action to folic acid, in the treatment of various malignant diseases, detected in the tissues of children with acute leukæmia, what he termed "an acceleration phenomenon in the leukæmic process". From this he deduced that related compounds or analogues which were antagonistic to folic acid, might be efficacious in the treatment of leukæmia by retarding the process. Subsequent to his report of temporary remission induced in the acute leukæmias of children by folic acid antagonists, it was decided to assess this method of therapy in children with leukæmia or allied conditions admitted to the Hospital for Sick Children.

DIAGNOSIS

In all cases treatment was withheld until the diagnosis was assured. The diagnosis was made on the basis of history, clinical findings, peripheral blood studies, x-ray studies of bone, marrow aspiration and occasionally lymph gland biopsy.

It becomes apparent, when a series of cases of leukæmic children were studied intensively, that each case has more or less individual characteristics. Whether these are variations in the same disease process involving a common type of stem cell, or whether they represent fundamental differences, possibly involving separate cell types, it is difficult to determine. In our experience, in most cases of leukæmia in children it is impossible to determine with any degree of assurance the type of cell from which the leukæmic cell is derived. In most cases of leukæmia in children the blast cell is so anaplastic that a diagnosis of the cell type based on minor cytological differences is not valid. Occasionally, one may hazard an opinion as to the type of leukæmic cell by the company it keeps, either in the peripheral film or the bone marrow aspiration. It may be a confession of ignorance on the part of the writers, but in most cases of leukæmia in children, our diagnosis was simply acute leukæmia.

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This study was made possible by a grant from the Ontario Cancer Treatment and Research Foundation.
The Folic Acid Antagonists were supplied by the Lederle Laboratories Division of The American Cyanamid Company.

METHODS OF TREATMENT

Fifty-three cases of leukæmia have been treated with folic acid antagonists. In 37, this was the only type of specific therapy attempted. In 16, the folic acid antagonists were used prior to or subsequent to treatment with ACTH or cortisone. The two antagonists used were aminopterin and a-methopterin. The former is approximately five times as toxic as the latter. As their effectiveness is proportional to their toxicity, there is little advantage in either chemical. The average dose of aminopterin was approximately 1 mgm. per day and that of a-methopterin 5 mgm. The dosage was continued until there was either an abatement of the leukæmia or signs of toxicity were evident. Both these effects usually appeared about the same time. In most patients a total of 7 to 10 mgm. of aminopterin or 35 to 50 mgm. of a-methopterin was given and then the treatment discontinued. These so-called "courses" were repeated as frequently as necessary until the tolerance of the patient was reached or it was obvious the leukæmic process was refractory to the chemicals. In some children an attempt was made to arrive at a maintenance dose but this was relatively unsuccessful in our hands. In two cases x-ray therapy was employed as well. In practically every patient, however, supportive treatment in the form of both direct and indirect transfusions and various antibiotics was liberally used. In the individual case, the use of supportive treatment, particularly blood transfusions might somewhat confuse the picture in regard to assessment of clinical or hæmatological improvement due to the drug therapy. However, as adjuvant treatment, particularly in children, is only of transitory benefit, any effect it may have had may be considered as inconsequential, and such effects have been taken into consideration in the evaluation of each case.

RESULTS

In appraising the effects of the folic acid antagonists in our series, it was found that according to their response, each patient might be placed in one of four main categories. This separation is of necessity arbitrary and there is in reality no sharp or definite division between the various types of response. The clinical and hæmatological criteria for each category are given below.

Category I.—This type of case temporarily responded dramatically to the administration of the folic acid antagonists. Following a latent period of four to seven days signs of general toxicity appeared. These consisted chiefly of increased irritability, lassitude and anorexia. Toward the end of this period a stomatitis might also develop which consisted of bleeding ulcers of various sizes on the gums, tongue, and buccal mucous membrane. After the first three to four days of treatment the total white blood count usually fell frequently to leukopenic levels with a relatively greater fall in the proportion of circulating blasts. At the same time the spleen, and lymph glands rapidly decreased in size. The administration of the chemical was usually stopped or the dose reduced because of the reduction of the white blood cells or signs of toxicity. By the end of the second week there was evidence of regeneration of the normal blood constituents. The reticulocyte count increased, immature but normal cells of the myeloid series began to appear in increasing numbers in the peripheral blood, and the platelet count which initially was usually at a low level approached normal.

The most important index of a remission was the state of the bone marrow, and in most of the cases 90% or more of the cells of the marrow at the time of the initial diagnosis were "blast forms" or primitive undifferentiated leukæmic cells. Concurrently with the improvement in the peripheral blood, the "blast forms" in the bone marrow were reduced to levels less than 4% and there was a flooding back of the normal marrow constituents. The marrow of these cases at the height of the remission could not be distinguished from a normal marrow. The child often resumed his normal activities and was to all appearances clinically normal. However, after a variable period of time usually from four to six weeks, there was a recurrence of the leukæmic picture in all its clinical and hæmatological aspects. After the first relapse, further treatment with the folic acid antagonists had much less effect. Attempts to arrive at a maintenance dose sufficient to keep the leukæmic process in abeyance was no more successful in our hands than an intermittent form of therapy. The number of patients in which such a clear-cut remission was produced, even though of short duration was a relatively small percentage of the total.

Category II.—These cases were similar to Category I, in that there was definite clinical

and hæmatological evidence of an abatement of the leukæmic process. But various manifestations of the disease still persisted. The enlarged lymph glands in some patients regressed in size but did not completely disappear. The enlarged spleen frequently became smaller but often remained palpable. Some blasts persisted in the peripheral circulation and although the blasts in the bone marrow decreased they were still present in excess of normal. Despite administration of the folic acid antagonists to the limit of the tolerance of the patient, a complete remission could not be obtained.

Category III.—These cases all showed the results of the toxicity of the drug and in addition the lack of an appreciable differential effect of the therapy on the leukæmic and the normal cells. At no time in these patients was there any evidence of clinical improvement that could not be explained on the basis of supportive therapy. After varying period of time and dosage, a rapid fall in the white blood count occurred with concomitant decrease in the size of spleen and peripheral lymph glands. The platelets, usually low at the onset, fell to still lower levels. Although the "blast forms" usually disappeared from the peripheral circulation, there was no evidence of regeneration of the normal blood constituents, and these patients, in spite of repeated transfusions and antibiotic therapy, died of multiple hæmorrhages from various sites and frequently generalized sepsis. Invariably at post-mortem, these children showed minimal evidence of the usual leukæmic infiltration except for possibly a few residuals in some of the lymph glands and the bone marrow. The latter was usually hypoplastic. There is little doubt that folic acid antagonists caused a marked regression on the leukæmic cells, but apparently the normal blood-forming tissue was at the same time so depressed that it was unable to regenerate.

Category IV.—In this type of case there was at no time any clinical hæmatological evidence that the therapy had any significant effect. Most of these patients received prolonged and intensive therapy, and the normal tissue as well as the leukæmia cells seemed to be more resistent to the toxic effects of the drug. All of these cases which came to post mortem showed the classical widespread infiltration with leukæmic cells which was so strikingly absent in many of our other treated cases. Apparently this type of reaction to the folic acid antagonists is more common in adults with leukæmia.

TABLE I.

LEUKÆMIA IN CHILDREN
FOLIC ACID ANTAGONISTS ONLY USED

Type of response	*Number of patients*
Category I.	4
Category II.	5
Category III.	17
Category IV.	11
Total.	37

In Table I are shown the results of 37 children in which the folic acid antagonists were the only specific therapy. In approximately 25% there was a partial or complete remission of varying duration. Fig. 2, is a graphic representation of the

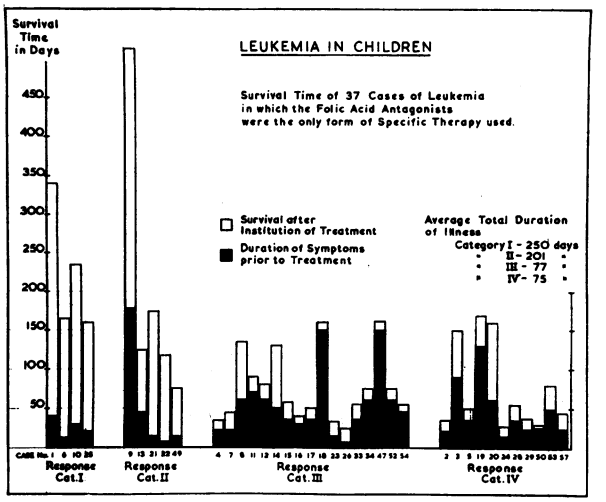

Fig. 2

disease process of this group of children with an approximation of the total length of their illness and the duration of life after the beginning of therapy.

The average expected survival of acute leukæmia in children not receiving specific therapy is less than 90 days.[3] It is considered that in category I and II, there was significant prolongation of life, as the average survival time of those with either a complete or partial remission was in excess of 200 days. Certainly in these 9 patients there was no case of chronic leukæmia and it is extremely unlikely that 25% of a series of leukæmia in children would be of the subacute type. Although our series of cases is too small for statistical analysis, it is our impression that the aleukæmic or subleukæmic type of leukæmia with a low or normal white blood count is relatively a more benign type of disease and responds better to specific therapy.

There is little doubt that treatment hastened the death in some of the patients falling into category III. Even though the total dosage was small, many of these patients, shortly after therapy was started, quickly passed into a profound hypoplastic phase in regard to both leukæmic cells and normal marrow constituents. The child usually succumbed in a few days from hæmorrhage and/or sepsis in spite of vigorous supportive therapy.

There is apparently a marked individual variation in susceptibility to the action of the folic acid antagonists. This effect apparently bears little relationship, except within wide limits to the age of the child, the duration, and the type of leukæmia. This is well demonstrated in the contrast between categories III and IV. In the latter the chemicals were frequently given in relatively large doses for long periods of time with only minor evidence of toxicity and no clinical or laboratory evidence of any effect on the leukæmic process, whereas in category III, relatively small amounts of the chemicals produced striking changes.

There was an additional group of 16 patients in which the folic acid antagonists were used either prior to or subsequent to treatment with ACTH or cortisone. In every case the sands had run out as far as the usefulness of the initial method of treatment was concerned. The switch to the other type of therapy, in addition to being obvious, was the only alternative. There was very little correlation between the response to the two types of treatment. Some cases responded well to the hormone and poorly to the chemicals and the converse was also true to some extent. An occasional patient was resistant to both forms of treatment. However, even in this small series of cases it is apparent from the results that a patient may become refractory to the continued use of either the folic acid antagonists or ACTH and cortisone and yet have a remission of considerable degree when the patient is changed to the alternative method of treatment. Thus, in some patients at least, the combined employment of the hormones and the folic acid antagonists may offer slightly more than will either form of therapy alone.

Table II is a summary of all the cases of leukæmia in which the folic acid antagonists were used. The category of response was judged entirely by the reaction of the patient to the antagonists and not to the effects of any previous or subsequent treatment with ACTH or cortisone.

TABLE II.

LEUKÆMIA IN CHILDREN
ALL CASES IN WHICH THE
FOLIC ACID ANTAGONISTS WERE USED

Type of response	*Number of patients*
Category I	4
Category II	9
Category III	23
Category IV	17
Total	53

DISCUSSION

From experimental work it appears that folic acid is necessary for the growth and multiplication of cells. Hence one would expect that the folic acid antagonists would be able to produce a relatively greater deficiency of folic acid in those cells which are undergoing rapid growth and multiplication. This probably accounts for their action on leukæmic cells and this effect is likely more of a relative than a specific or selective effect. The failure of the folic acid antagonists to have any appreciable effect on the chronic leukæmias of adults and a significant proportion of leukæmias in children could be explained on the basis that in these cases the differential between the effects of the folic acid antagonists on normal cells and leukæmic cells was not sufficient to produce any appreciable effect on the leukæmic process before the overall toxicity of the folic acid antagonists manifested itself. One argument against the assumption that the folic acid antagonists produce a folic acid deficiency is that in none of our cases was there any evidence of the development of a macrocytic or megaloblastic type of reaction in the erythroid elements which is regarded as one of the features of folic acid deficiency.

Whatever is the mode of action of these drugs, there is no doubt that in some of the acute leukæmias of children a dramatic temporary, clinical and hæmatological remission can be produced. These remissions were not sustained for any appreciable period of time and subsequent relapses were less amenable to treatment. In some cases, judging by the findings in the peripheral blood and the bone marrow, one appears to be able to keep the leukæmia under control with continued therapy, but the normal hæmopoietic tissue seems to lose its ability to regenerate and the

patient finally succumbs because of an insufficient supply of normal leukocytes and platelets.

Obviously these chemicals can in no way be considered as a cure for leukæmia, and from a practical point of view, they cannot even be considered as a very satisfactory form of therapy. However, in a few cases their use has definitely produced a remission and prolonged the life of the patient. During these remissions the patient had a relatively normal and comfortable existence for a limited period of time.

The ultimate failure of this type of treatment should not be cause for depreciation nor despair, but rather it should be considered as an important step in our understanding of this disease. It is an indication that the control of a malignant cell may be possible by interference with its metabolism. As information accumulates concerning the metabolism of normal and malignant cells, perhaps fundamental differences, either qualitative or quantitative will be uncovered. In such an event it is quite possible that suitable inhibitors to malignant cells may be found. As this has been achieved to a remarkably successful degree with regard to infectious agents, it is hoped that ultimately the same success may be attained with regard to neoplastic diseases.

CONCLUSIONS

1. The folic acid antagonists aminopterin and a-methopterin were used in the treatment of 53 cases of leukæmia in children.

2. In no case was a permanent cure obtained, but in 13 patients or 26%, there was a complete or partial clinical and hæmatological remission of short duration with some prolongation of life.

3. Except for transient episodes of toxicity, in general those patients which responded favourably were maintained in reasonably good health until shortly before death.

4. The development of a refractory state to the administration of the folic acid antagonists did not preclude the possibility of a remission with an alternative type of treatment.

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ACTH AND CORTISONE IN THE TREATMENT OF LEUKÆMIA IN CHILDREN

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IT IS NOT WITHIN THE SCOPE of this communication to review or recapitulate the various physiological and biochemical phenomena associated with the exhibition of the hormones ACTH and cortisone. By now it is well known that the administration of either ACTH or cortisone can bring about profound alterations in the total metabolism and cellular reactions of an individual in such a manner that the usual course of many diseases is dramatically modified or even temporarily reversed.

Our interest in the possible use of these drugs in the treatment of leukæmia was first aroused in 1949. As there was an accumulation of experimental data in both man and animals that activity of the adrenal cortex had a depressing effect on lymphoid tissue, lymphocytes, and eosinophils, there was some indication that this was a rational method of approach. The temporary but apparently complete remission induced in a patient with eosinophilic leukæmia by ACTH at the Hospital for Sick Children[1] and word-of-mouth reports concerning favourable responses with similar forms of treatment in other centres stimulated us to further investigate the possibilities of this type of therapy.

METHODS

The diagnosis of leukæmia was made on the basis of history, physical examination peripheral blood, bone marrow aspiration and occasional biopsy. Prior to and during treatment daily blood examinations including absolute eosinophile counts were done. Repeated marrow aspirations were performed at approximately weekly intervals depending upon the apparent clinical and hæmatological progress of the patient. All the patients in addition to the hormone therapy received as adjuvants

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This study was made possible by Grants from the Ontario Cancer Treatment and Research Foundation and the National Research Council of Canada from funds made available by the Department of National Health and Welfare from National Health Grants.